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REVIEW ARTICLE

Genetic Markers and Danger Signals in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Wen-Hung Chung[1] and Shuen-Iu Hung[2]

ABSTRACT

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse reactions, which could be induced by a variety of drugs. It was proposed that human leukocyte antigen (HLA)-restricted presentation of antigens (drugs or their metabolites) to T lymphocytes initiates the immune reactions of SJS/TEN. However, the genetic susceptibility and the exact pathogenesis were not clear until the recent studies. We first identified that HLA-B*1502 is strongly associated with carbamazepine (CBZ)-induced SJS/TEN and HLA-B*5801 with allopurinol-SJS/TEN in Han Chinese. The same associations had been validated across different human populations. For the downstream danger signals, Fas-Fas ligand (FasL) and perforin/granzyme B had been advocated as cytotoxic mediators for keratinocyte death in SJS/TEN. However, expression levels of these cytotoxic proteins from the skin lesions were too low to explain the distinct and extensive epidermal necrosis. Our recent study identified that the granulysin, a cytotoxic protein released from cytotoxic T cells or natural killer (NK) cells, is a key mediator for disseminated keratinocyte death in SJS/TEN. This article aims to provide an overview of both of the genomic and immunologic perspectives of SJS/TEN. These studies give us a better understanding of the immune mechanisms, biomarkers for disease prevention and early diagnosis, as well as providing the therapeutic targets for the treatments of SJS/TEN.

KEY WORDS

drug hypersensitivity, genetic polymorphism, NK cells, Stevens-Johnson syndrome, toxic epidermal necrolysis

INTRODUCTION

Drug hypersensitivity is a major clinical problem. Among the many types of drug hypersensitivity, SJS and TEN are the most serious and life-threatening adverse reactions. According to the clinical presentation and immunohistochemistry data, SJS and TEN have been considered as an immune disorder, involving both the adaptive and innate immune reactions. Applying techniques of pharmacogenomics and molecular biology in recent studies further revealed that the genetic disposition as well as immune mediators are important for the development of SJS and TEN. Although Fas-FasL interaction was previously considered to be the main effector in triggering apoptosis of keratinocytes, recent evidence suggested that granulysin, a cytotoxic protein produced by cytotoxic T lymphocytes (CTLs) and NK cells, is the one actually "turns on" the extensive apoptosis in keratinocytes.[1] In this article, we review the genomic and immunologic perspectives of SJS/TEN.

CLINICAL MANIFESTATIONS OF SJS/TEN

SJS/TEN are life-threatening severe cutaneous adverse reactions, presenting 10-40% mortality rate. SJS and TEN are classified as the same disease with different spectrums of severity according to the magnitude of epidermal detachment.[2] Early symptoms of the abrupt onset of SJS usually start with fever, sore throat, and malaise, following by rapidly developing blistering exanthema of macules and target-like lesions accompanied mucosal involvement with less than 10% of skin detachment.[2] TEN has similar clinical presentations with a more extensive separation of large sheets of epidermis from the dermis (greater than 30%) and a higher mortality rate (30-40%).[3] The

[1]Department of Dermatology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University and [2]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan.
Correspondence: Wen-Hung Chung, Department of Dermatology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, 199 Tung-Hwa North Road, Taipei 105, Taiwan.
Email: chung1@cgmh.org.tw
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skin lesions usually begin on the trunk and spreads proximally in both of the SJS and TEN patients.[2,3]

Although having a low incidence, complications and its sequelae of SJS/TEN can results in death or disability in previously healthy people.[4] Patients experiencing SJS/TEN are affected irreversible mucosal damage and the most serious ocular sequelae. Synechiae, corneal ulcers, symblepharon, and xerophthalmia are frequent ocular complications during the progression of SJS/TEN,[5] and photophobia and xerophthalmia are commonly developed eye sequelae affected SJS/TEN survivals lifelong.[6] Other internal organ involvements at mucosal surfaces are also common in SJS/TEN. With the increasing severity, abnormalities in respiratory tract,[7] gastrointestinal tract,[8] liver, and/or kidney are occasionally reported.[9]

The histopathology findings show that the significant feature of large portion epidermis separation from dermis is induced by massive keratinocyte apoptosis in SJS and TEN.[10] Although Fas-FasL interaction was previously considered to be the main effector in triggering apoptosis of keratinocytes, evidence suggests that the granulysin1 is the one actually "turns on" apoptosis of keratinocyte.[11] The main purpose of this review is to elucidate the pathomechanism of SJS and TEN in genetic and immunologic point of views.

GENETIC MARKERS RELATED TO SJS/TEN

Genetic association between HLA alleles and SJS/TEN was found from several reports, and the ethnicity specific feature is emphasized these years. A significantly strong correlation was first found in Han Chinese in 2004.[12] CBZ-induced SJS/TEN patients carried 100% of HLA-B*1502 allele, and only 3% HLA-B*1502 carriers tolerated CBZ.[12] According to its absence in Caucasians[13] and Japanese,[14] HLA-B*1502 allele seems uniquely limited in Han Chinese ancestral Asians and might be an explanation for the extremely high risk of CBZ-induced SJS/TEN in Southeast Asians comparing to Caucasians and Japanese. Later studies conducted in the South-East countries, including Hong Kong, Malaysia, India, Singapore, Thailand, Vietnam, Indonesia, and Philippines, were further confirming its high prevalence (2.3% to 8.4%) of this ethnic specific allele.[15] Because of the ethnic specialty in HLA-B*1502, CBZ and some similar structural anticonvulsants were fully studied. One hundred percent CBZ-induced SJS was HLA-B*1502 in Thailand people.[16] Malay and Chinese carried 15.7% and 5.7% of HLA-B*1502, respectively, and had 75% incidence of HLA-B*1502 in CBZ-SJS or TEN.[17] A lower prevalence (2.5%)[18] and 75% incidence of HLA-B*1502 in CBZ-induced SJS were reported in Indians.[19]

Other than HLA-B*1502, HLA-B*5901 has been suggested as a candidate marker in CBZ-induced SJS in Japanese with 15.16 relative risk[20]; however, due to its small sample size (10 patients), further investigation is needed to validate this finding. In addition, HLA-A*0206, was proposed as a marker in SJS/TEN according to the ocular complications in Japanese.[21]

HLA-B*5801 allele is another genetic marker found to have an association with a commonly prescribed drug for decreasing uric acid level in hyperuricemia—allopurinol. In Taiwanese, it was 100% presented in patients with allopurinol-induced severe cutaneous adverse reactions, but only 15% in allopurinol tolerant patients.[22] The same strong association was confirmed in a Thai population.[23] However, findings from Japanese population remain to clarify. A moderate association (4 out of 10) in a Japanese population[14] was reported. Opposite to this finding, Kano *et al.* suggested that HLA-B*4801 but not B*5801 was associated with CBZ-induced drug rash with eosinophilia and systemic symptoms (DRESS).[24] In addition, the ethnic limitation also presents in HLA-B*5801 on its low allele frequency in Caucasians.[25]

Moreover, an antiretroviral drug, abacavir, can cause drug hypersensitivity and was linked to HLA-B*5701 allele. The combination of HLA-B*5701, HLA-DR7, and HLA-DQ3 once reported having a 100% predictability of the abacavir-induced hypersensitivity.[26] Further study demonstrated that the prevalence of HLA-B*5701 was high in Caucasians and suggested that prior screening of the carriage of HLA-B*5701 could effectively reduce the abacavir hypersensitivity.[27] In contrast to the high prevalence of HLA-B*5701 and incidence of abacavir hypersensitivity, none of the patients with Asian ancestry in Korea carried HLA-B*5701.[28]

DRUG-SPECIFIC, HLA-DEPENDENT, T-CELL-MEDIATED IMMUNITY IN SJS/TEN

The discoveries of the involvement of HLA-dependent presentation of an offending drug or its metabolites for T-cell activation[29,30] demolished the "hapten hypothesis"[31] in severe drug hypersensitivity. The proposed pharmoco-immune (p-i) concept[32] is the mainstream explanation for the drug-induced delay cutaneous adverse reactions, suggesting the direct and non-covalent binding between a drug and T-cell receptors (TCR) with HLA molecules takes the responsibility for the drug-induced immunity (Fig. 1). The pathogenesis of the induction of cytotoxic responses in SJS/TEN is generated by the recognition of offending drugs to HLA class I molecule initiated T-cell activation which results a clonal expansion of CD8+ cytotoxic T-cells in skin.[33] Our findings, the strong associations between HLA-B*1502 and CBZ[12] as well as the HLA-B*5801 and allopurinol,[22] support that drug-induced SJS/TEN is a HLA-restricted immunity.[12] Furthermore, our later results verified 5 peptides showing high affinities for HLA-B*1502, providing CBZ recognition of HLA, locating at antigen presenting cells.[34,35]

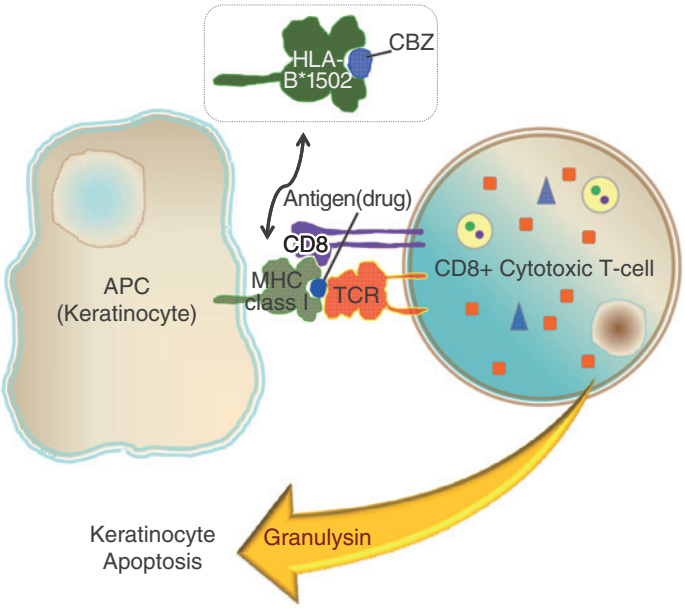

Fig. 1 A model of keratinocyte apoptosis induced by the immune synapse of drug-HLA-TCR interaction in SJS/TEN. As illustrated, the immune response may be triggered by the binding of an antigenic drug (e.g. carbamazepine [CBZ]), to a specific HLA allele (e.g. HLA-B*1502) on a keratinocyte, which are the main antigen presenting cells (APC) in SJS/TEN. Then, specific T cell receptors (TCR) of the CD8+ cytotoxic T lymphocytes (CTLs) recognize the drug-HLA complex. Upon the activation, CTLs or NKT cells produce cytokines and chemokines, as well as the cytotoxic proteins, particularly, secretory granulysin and lead to extensive keratinocyte apoptosis.

INVOLVEMENT OF NATURAL KILLER CELLS IN SJS/TEN

In addition to CTLs, NK cells also involves in SJS/TEN. The blister cells in skin lesions of SJS/TEN-affected patients were mainly CTLs and NK cells[36]; moreover, HLA class I-binding proteins have activating (KAR, killer activating receptor) or inhibitory (KIR, killer inhibitory receptor) cytolytic functions for regulating NK cells.[37] Nassif A. *et al.*[29] further demonstrated an observation of the infiltration of CTLs and NK cells in skin lesions in TEN patients. Our recent study also demonstrated that granulysin, secreted from CTLs and NK cells, is a key effector responsible for keratinocytes death in SJS/TEN.[1]

DANGER SIGNALS INDUCING EXTENSIVE KERATINOCYTE APOPTOSIS IN SJS/TEN

Upon proposed immunopathogenesis of SJS/TEN, the general agreed central hypothesis is the T-cell-mediated massive apoptosis in keratinocytes. Until recently, there are three reported pathways advocated as basic effectors: Fas-FasL interaction, perforin/granzyme B, and the granulysin.

Fas-FasL-INDUCED APOPTOSIS

Although Fas-FasL-induced apoptosis in keratinocytes is one of the most thoroughly studied immune mechanism in SJS/TEN, inconsistent findings of Fas, FasL, and soluble FasL (sFasL) questioned the original hypothesis from Viard *et al.*[11]

Fas was discovered to cause cell death upon binding with its ligand in SJS/TEN. Viard *et al.*[11] suggested the activated Fas servers as a death receptor in triggering apoptosis of keratinocytes in SJS/TEN. The cytoplasmic death domain of Fas undergoes conformational changes upon recognition of FasL. The Fas-FasL then recruits a Fas-associated death domain protein (FADD) which has a affinity to bind to both of the Fas death domain and procaspase 8. Once the procaspase 8 is recruited by FADD, the multiple copies of procaspase 8 are brought together and autoactivate themselves to caspase 8 which triggers the caspase cascade for intracellular DNA degradation.[38] One study concluded the same result as Viard *et al.* by incubating cell-free supernatants of blister fluid containing sFasL secreted from keratinocytes and not resulting in apoptosis in keratinocytes.[39]

Viard *et al.* also showed that FasL presented on the

cell surface of keratinocytes in TEN patients and sFasL was found to have high levels in the serum, but not in patients with maculopapular drug reaction or normal persons.[11] Metalloproteinases (MPs) present at cell surface in many tissue types, including keratinocytes[40] and cleave FasL into sFasL at its TNF-homologous portion.[41,42] The high sFasL serum level observed in TEN patients might cause by the MP cleavage reaction. A study also consistently found an apparent elevation of sFasL within 2 days after the onset of skin damage in a TEN patient.[43] Testing the serological sFasL for a short period at the beginning of onset may help to define the progress of SJS/TEN.

Controversial interpretations disagreed with either the source of FasL or its role in apoptosis effector. Studies demonstrated that FasL is not located at the extracellular membrane surface of keratinocytes, but rather transporting to the cell surface upon suffering keratinocyte damage.[40,44] Abe *et al*.[45] found that the apoptosis of cultured keratinocytes was induced by adding high levels of sFasL containing sera isolated from a SJS/TEN patient, and was blocked by addition of anti-FasL monoclonal antibody. They also showed the peripheral blood mononuclear cells (PBMCs) of TEN patients were the sites produced high levels of sFasL.[45]

Since Amato *et al*.[46] first reported the treatment of intravenous immunoglobulins (IVIG) in a SJS patient, many studies subsequently applied IVIG to treat the SJS and TEN patients. IVIG therapy is a treatment based on the hypothesis of Fas-FasL interaction, by blocking FasL binding to the Fas receptor interferes with the downstream signaling for triggering apoptosis of the keratinocytes.[11] Although evidence seemed to show the effectiveness of IVIG in *in vitro* studies,[11,47] benefits were inconsistently seen in neither the mortality nor the progression of skin detachment,[48-52] which yield the controversial role for the IVIG treatment in SJS/TEN.

PERFORIN/GRANZYME B APOPTIC PATHWAY

High concentrations of granzyme B was found in TEN blister fluid.[53] Nassif A. *et al*.[54] presented a contradictory hypothesis to the Fas-FasL interaction by showing that the cytotoxicity from the blister fluid mononuyclear cells in TEN could be blocked by inhibitors of perforin/granyzyme B rather then blocked by an anti-Fas monoclonal antibody. Perforin and granzyme B are stored in secretory granules of activated CTLs and NK cells.[55] Perforin binds and punches a channel in the membrane of target cells for entering of the granzyme B to activate the caspase cascade and its following apoptotic pathways.[56,57]

OTHER CYTOKINES AND SIGNALS INVOLVED IN THE PATHOGENESIS OF SJS/TEN

In addition to Fas-FasL or perforin/granzyme B pathways, some cytokines, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ,[39] and interleukin (IL)-10,[39] were also found to be up-regulated in SJS/TEN. Blister cells of SJS/TEN were reported to secret IFN-γ and stimulate keratinocytes to express TNF-α, FasL, and IL-10 which were present in higher concentrations in the blister fluids as a defense mechanism against CTLs.[39] TNF-α has been reported abundantly presenting in the keratinocytes of epidermis,[58,59] blister fluid mononuclear cells and macrophages[53,58,59] and PBMCs[60] in SJS/TEN. TNF-α has been suggested having an up-regulating function in Fas and FasL[60] and via the activation of TNF-receptor 1 (TNF-R1), initiating the downstream FADD and caspases.[61,62] Apoptosis activation in TEN patients had been implied to be induced by TNF.[10] In addition, little evidence showed that TNF-α also can activate TNF-related apoptosis-inducing ligand (TRAIL) and its receptors (TRAIL-Rs), which may result in the activation of FADD apoptotic pathway.[63] Interestingly, contradictory to TNF-α apoptosis inducing role, its binding to TNF-R1 can up-regulate NF-κB[64] and associate with an anti-apoptotic in keratinocytes.[65] Anti-TNF therapy once was thought to be a possible potential treatment for TEN patients; however, no beneficial effect was concluded from anti-TNF by thalidomide in TEN.[66]

There was one study that supported both of the Fas-FasL and perforin/granzyme B pathways in keratinocytes apoptosis.[53] This study found an increasing pattern of TNF-α, perforin, granzyme B, and FasL accompanied with the severity, ranging from the mildest maculopapular rashes to the severest TEN, and suggested that the perforin/granzyme B played as the major effectors and Fas-FasL interaction was probably related to the severer adverse drug reactions.

GRANULYSIN IS THE MAJOR FACTOR FOR KERATINOCYTE APOPTOSIS IN SJS/TEN

Recently, we found secretory granulysin servers as a major cytotoxic molecule responsible for widespread keratinocyte necrosis in SJS/TEN,[1] instead of those previously reported sFasL, granzyme B, or perforin. We performed global gene expression profiling of the blister cells and found that granulysin RNA was the most significant cytotoxic molecule expressed. Granulysin protein concentrations in the SJS/TEN blister fluids were two to four orders of magnitude higher than perforin, granzyme B or sFasL concentrations, and depleting granulysin reduced the cytotoxicity (Fig. 2). Westernblot analysis showed that granulysin in the fluids was predominantly the 15-

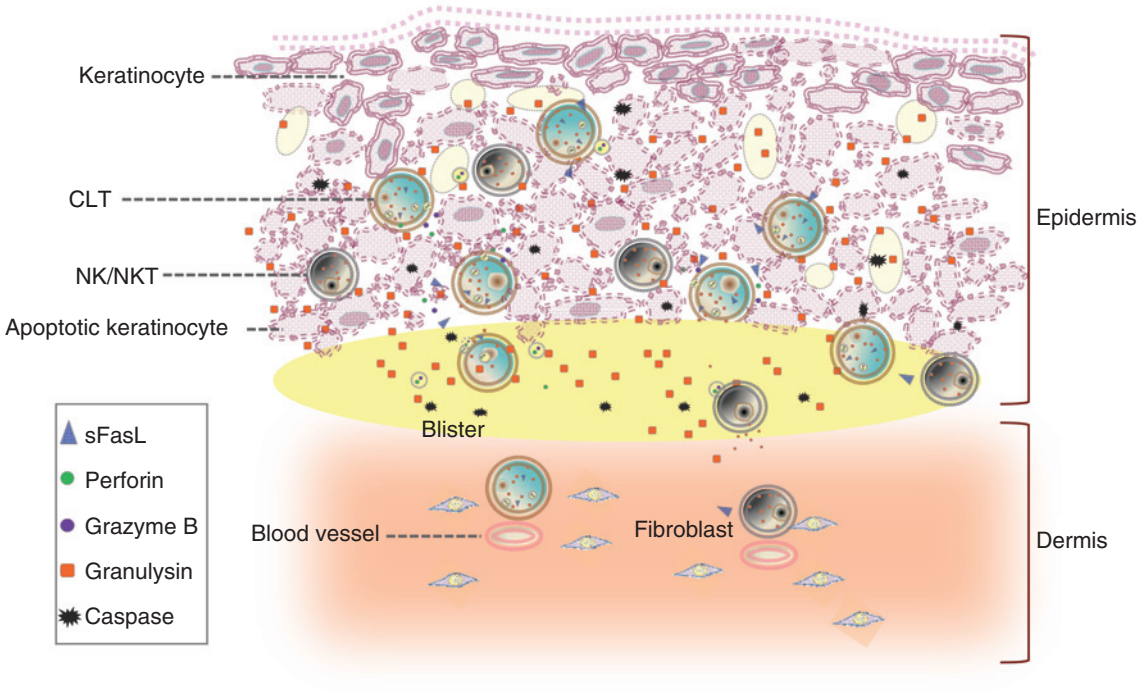

Fig. 2 Pathogenesis of epidermal necrosis and disseminated keratinocyte apoptosis in SJS/TEN. Due to the signals of immune synapse, the CTLs and NK/NKT cells immigrate to the epidermis of skin. CTLs, and NK/NKT cells produce a large amount of immune mediators (e.g., soluble FasL [sFasL], perforin, grazyme B, and granulysin) into the extracellular space. In particular, secretory granulysin, expressed at a very high level in the skin lesions, is a main weapon of CTLs, NK, and NKT cells, attacks keratinocytes and results in extensive epidermal necrosis and blister formation. By comparison, granzyme B/perforin and Fas/FasL are produced via granule exocytosis upon cell-cell contact, and present in lower concentrations than granulysin in the inflammatory lesions. After encountering the attacks of these cytotoxic proteins, keratinocytes are damaged and then the caspase signaling pathway turns on, leading to apoptosis progress.

kDa secretory form. *In vitro*, purified 15-kDa granulysin exhibited significant cytotoxicity at the level presenting in the SJS/TEN blister fluids. However, sFasL, perforin, and granzyme B concentrations in the SJS/TEN blister fluid had minimum cytotoxicity. A further injection of granulysin into mouse skin resulted in a SJS-TEN-alike skin necrosis.[1] Thus, our findings demonstrated that granulysin, not granzyme B, perforin or sFasL as previously implicated, is the key molecule responsible for the disseminated keratinocyte death in SJS/TEN. Following this line, Abe *et al.*[45] further reported that an increasing serum level of granulysin could serve as an early diagnostic biomarker for SJS/TEN.

Granulysin is a cationic cytolytic protein produced by CTLs, NK and NKT cells.[67] The 15-kDa granulysin was proposed as a precursor of the 9-kDa form.[68,69] In addition to having the cytotoxic effect, granulysin also was demonstrated its chemoattractant ability for T lymphocytes, monocytes and other inflammatory cells, and activation function in the expression of a number of cytokines, including RANTES/CCL5, MCP-1, MCP-3, MIP-1α/CCL3, IL-10, IL-1, IL-6 and IFN-α.[70]

However, much of SJS/TEN remains in mystery. How does taking a drug lead to secretion of granulysin? How does CD8+T/NK and NKT cells regulate the secretion of granulysin in SJS/TEN? The specific relationship among offending drugs, HLA alleles, and cytotoxic signals from CLTs/NK/NKT cells in SJS/TEN remains further investigation.

CONCLUSION

Increasing data have revealed that the genetic predisposition and immune mediators play important roles in the drug hypersensitivity. As reviewed in this article, it is known that HLA alleles associated with SJS/TEN may be variable among different human population. These HLA alleles may be not only responsible for the genetic susceptibility, but also play a pathogenesis role, which may present the drugs/metabolites to CTLs for the initiation of the downstream danger signals in the disease. In addition to Fas-FasL, perforin/granzyme B biomarkers, the secretory 15 kDa granulysin, is now known to be the major weapon of CTLs/NK/NKT cells and leads to the ex-

tensive epidermal necrolysis in SJS/TEN. Understanding the molecular mechanism of the interaction of HLA, offending drugs and TCR, as well as CTLs/NK cells activation, would facilitate the development of new approaches for the management of SJS/TEN. In conclusion, those transitional studies offer us a better understanding of the immune mechanisms, biomarkers for disease prevention and early diagnosis, as well as providing the therapeutic target for the treatment of SJS/TEN.

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